CLAIMS

1. A method of amplifying, and optionally also detecting, a target nucleic acid sequence, the method comprising the steps of:

a) providing a sample that may or may not comprise a target nucleic acid sequence,

b) providing a pair of outer primers and a pair of inner primer, a nucleic acid polymerase and standard reagents for PCR, the melting temperature (Tm) of the pair of outer primers being at least 2 °C higher than the Tm of the pair of inner primers,

c) contacting the sample with the pair of outer primer and the pair of inner primers, and standard reagents for PCR, thus obtaining the reaction mixture,

d) cycling, at least two times, the temperature of the reaction mixture between a first denaturation temperature, a first annealing temperature and a first extension temperature, the first annealing temperature being similar to or lower than the lowest Tm of the outer primer pair and higher than the highest Tm of the inner primer pair,

e) cycling, at least two times, the temperature of the reaction mixture between a second denaturation temperature, a second annealing temperature and a second extension temperature, the second annealing temperature being similar to or lower than the lowest Tm of the inner primer pair,

f) optionally, analysing the product of step d and/or step e) to detect the presence of the target nucleic acid sequence.

2. The method according to claim 1, wherein at least one primer of the outer primer pair comprises a Tm-increasing component.

3. The method according to claim 1 or 2, wherein both of the primers of the outer primer pair comprise a Tm-increasing component.

4. The method according to claim 3, wherein the Tm-increasing component binds non-specifically to nucleic acids.

5. The method according to any of claims 2-4, wherein the Tm-increasing component comprises one or more moieties selected from the group consisting of a modified nucleotide and a minor groove binding agent.

6. The method according to claim 5, wherein the modified nucleotide is a peptide nucleic acid (PNA) or a locked nucleic acid (LNA).

7. The method according to any of claims 2-6, wherein the Tm-increasing component increases the Tm of the primer with at least 1°C relative to the Tm of the same primer not comprising the Tm-increasing component.

8. The method according to any of the preceding claims, wherein the second denaturation temperature is at least 1°C lower than the first denaturation temperature.

9. A method for detection of *Bacillus anthracis*, the method comprising detecting a target nucleic acid sequence according to the method of claim 1-8, the target nucleic acid sequence being specific for the pXO1 or pXO2 plasmid of *Bacillus anthracis,* wherein the pair of outer primers and the pair of inner primers are selected from the pXO1 or pXO2 plasmid of *Bacillus anthracis*.

10. The method according to claim 9, wherein the pair of outer primers and the pair of inner primers are selected so as to amplify a target nucleic acid sequence related to a gene selected from the group of *B. anthracis* genes consisting of capA gene, the capB gene, the capC gene, the lef gene.

11. The method according to claim 9 or 10, wherein target nucleic acid sequence is related to the capA gene and

- a primer of the pair of outer primers comprises a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 2, a homologous sequence thereof, and a complementary sequence thereof, and
- a primer of the pair of inner primers comprises a nucleic acid sequence selected from the group of SEQ ID NO: 3, SEQ ID NO: 4, a homologous sequence thereof, and a complementary sequence thereof.

12. The method according to any of the claims 9-11, wherein target nucleic acid sequence is related to the capA gene and the pair of outer primers comprises SEQ ID NOs: 1 and 2 and/or the pair of inner primers comprises SEQ ID NOs: 3 and 4.

13. A kit comprising a pair of outer primers and a pair of inner primer, the melting temperature (Tm) of the pair of outer primers being higher than the Tm of the pair of inner primers.

14. The kit according to claim 13, wherein at least one primer of the outer primer pair comprises a Tm-increasing component.

15. The kit according to claim 13, wherein both of the primers of the outer primer pair comprises a Tm-increasing component.

16. The kit according to any of the claims 13-15, wherein the Tm-increasing component binds non-specifically to nucleic acids.

17. The kit according to any of the claims 13-15, wherein the Tm-increasing component comprises one or more moieties selected from the group consisting of a modified nucleotide and a minor groove binding protein.

18. The kit according to claim 17, wherein the modified nucleotide is a peptide nucleic acid (PNA) or a locked nucleic acid (LNA).

19. The kit according to any of the claims 13-18, wherein the Tm-increasing component increases the Tm of the primer with at least 1°C relative to the Tm of the same primer not comprising the Tm-increasing component.

20. Kit for detection of *Bacillus anthracis*, the kit comprising a pair of outer primers and a pair of inner primer, the melting temperature (Tm) of the pair of outer primers being higher than the Tm of the pair of inner primers, wherein the pair of outer primers and the pair of inner primers are selected from the pXO1 or pXO2 plasmid of *Bacillus anthracis*.

21. The kit of claim 20, wherein the pair of outer primers and the pair of inner primers are selected so as to amplify a target nucleic acid sequence within a gene selected from the group of *B. anthracis* genes consisting of capA gene, the Cap B gene, the Cap C gene, the lef gene.

22. The kit according to claim 20 or 21, wherein target nucleic acid sequence is related to the capA gene and

- a primer of the pair of outer primers comprises a nucleic acid sequence selected from the group of SEQ ID NO: 1, SEQ ID NO: 2, a homologous sequence thereof, and a complementary sequence thereof, and
- a primer of the pair of inner primers comprises a nucleic acid sequence selected from the group of SEQ ID NO: 3, SEQ ID NO: 4, a homologous sequence thereof, and a complementary sequence thereof.

23. The kit according to any of the claims 20-22, wherein target nucleic acid sequence is related to the capA gene and the pair of outer primers comprises SEQ ID NOs: 1 and 2 and/or the pair of inner primers comprises SEQ ID NOs: 3 and 4.

24. An analysis system for detection of a microorganism, the analysis system comprising a pair of outer primers and a pair of inner primer, the melting temperature (Tm) of the pair of outer primers being higher than the Tm of the pair of inner primers.

25. The analysis system of claim 24, wherein the analysis system is selected from the group consisting of a lateral flow device, a biochip, and a microarray.